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ABSTRACT

Introduction. The present research explores data and analyzes molecular mechanisms appraisal of the clinical course of chronic viral hepatitis C, registered considering the prognostic significance of the CYP2C9 isoenzyme. **The aim of the research** is to assess the molecular mechanisms of the clinical course of CVHC, considering the prognostic significance of the CYP2C9 isoenzyme, responsible for the xenobiotic biotransformation system, in representatives of the Uzbek population. **Materials and methods**. 107 patients with chronic viral hepatitis C (CVHC) were involved in the research group, further were divided into three subgroups to assess the association of polymorphic markers of the CYP2C9 genes. The criteria for inclusion in the research were clinical, biochemical and instrumental verification of the diagnosis with the determination of both the stage and severity of the disease, as well as the detection of hepatitis C virus RNA by the AmpliSens® HCV-FRT test system detected by polymerase chain reaction (PCR) on a RotorGene 6000 instrument. **Results and discussion.** Research data determined that a relationship was established between the mutant allele of CYP2C9 gene polymorphism with more favorable course of CVHC. This assumption is also confirmed by the reduced frequency of the mutant allele in the subgroup of patients with highly active CVHC, which may indicate the protective role of this allele in activating the inflammatory process in CVHC. It was revealed that a high level of theoretically

expected heterozygosity is the indicator of genetic diversity in the subgroup of patients with high active CVHC. **Conclusion.** The results of the research suggest that CYP2C9 gene polymorphism can be considered as a conditionally "protective" marker for disease progression and requires further study in a larger sample of patients.

Key words: chronic hepatitis, pathogenesis, cytochrome P450, isoenzyme CYP2C9, molecular genetic research, gene polymorphism

Introduction

To date the increase in prevalence and development of chronic viral hepatitis C (CVHC) complications remains at the same level both in the developed countries and Uzbekistan (1). In addition, the fact that CVHC is one of the main reasons for the development of liver cirrhosis and hepatocellular carcinoma remains an important aspect (2, 3, 10). It should be noted that in approximately 22% of cases, the chronic form of hepatitis C leads to cirrhosis of the liver (4, 11). In connection with the foregoing, the issue of the prognosis of morbidity is of particular importance. This aspect is considered to be quite relevant and urgent in our region.

Recently, much attention of specialists has been caused by the possibility of a personalized approach to the diseases treatment based on human genetic characteristics (13). CYP2C9 is one of the necessary significant isoenzymes of cytochrome P450 in the liver (9). It should be noted that the content of the CYP2C9 isoenzyme in the liver is 18%, determining its significance in the development of liver diseases (5, 12).

The limited data on exploring of the Uzbek population determined the relevance and need for research to identify allelic variants of candidate genes associated with the characteristics of the course of CVHC.

The aim of the study is to assess the molecular mechanisms of the clinical course of CVHC, considering the prognostic significance of the CYP2C9 isoenzyme, responsible for the xenobiotic biotransformation system, in representatives of the Uzbek population.

Material and methods. The main group included 107 patients with CVHC. To assess the association of polymorphic markers of the CYP2C9 genes, patients with CVHC were divided into three subgroups. The first subgroup included patients with a moderate degree of CVHC activity (n=33). The second subgroup consisted of patients with a high degree of CVHC activity (n = 37). The third subgroup included patients with cirrhosis (n=37). The criteria for study inclusion were clinical, biochemical and instrumental verification of the diagnosis with the

determination of both the stage and severity of the disease, as well as the determination of hepatitis C virus RNA by the AmpliSens® HCV-FRT test system identified by polymerase chain reaction (PCR) on a Rotor Gene 6000 instrument.

As a comparison group, a population control was used, represented by DNA samples (n = 81) of conditionally healthy donors (without chronic hepatitis C) from the DNA bank of the Research Institute of Hematology and Blood Transfusion of the Ministry of Health of the Republic of Uzbekistan.

The material for molecular genetic research was the peripheral blood of the patients. DNA isolation was carried out according to the standard method with some modifications and using reagents of the company Interlabservis (Russia). Alleles of gene polymorphism were identified using PCR.

Results and Discussion. The scientific research on the frequency of alleles has shown that in both groups of patients and in the group of population control, the wild-type allele was predominant. Moreover, in patients with chronic hepatitis C, the frequency of occurrence of the “wild” allele (C) of CYP2C9 polymorphism was 86.9%, and the mutant allele (T) was 13.1%. The revealed values of the allele frequency in the general group of patients did not practically differ from the data of the indicators of the studied population (87.6% and 12.3%, respectively, $\chi 2 = 0.05$; P = 0.83; OR = 1.07; 95% CI 0.58-1.97). Some differences in the indicators were revealed in the study of patient samples in accordance with the stage and activity of the pathological process. A similar control value for the mutant allele frequency was observed in the subgroup of patients with cirrhosis (12.2%; $\chi 2 = 0.002$; P = 0.97; OR = 0.98; 95% CI 0.42-2.28). Simultaneously, an increase in the frequency of occurrence of the T allele was noted in the subgroup of patients with high active CVHC (19.7%; $\chi 2 = 2.05$; P = 0.15; OR = 1.74; 95% CI 0.81- 3.75), and the lowest indicator of the frequency of the mutant allele was observed in the second group of patients at the peak of the activity of the pathological process (8.1%; $\chi 2 = 0.002$; P = 0.97; OR = 0.98; 95% CI 0 , 42-2.28). Moreover, the difference in the incidence rate of the mutant allele was significant both between the first and second, and between the second and third ($\chi 2 = 3.99$; P = 0.05; OR = 2.78; 95% CI 0.9907-7.801) subgroups of patients. The increased frequency of the T allele in the group of patients with high active chronic hepatitis C is evidence of its possible association with an inactive course of the disease. This assumption is also proved by the reduced frequency of the mutant allele in the subgroup of patients with highly active CVHC, which may indicate the protective role of this allele in relation to the activation of the inflammatory process in CVHC. Thus, there is a clear connection between the frequencies of occurrence of the mutant allele with a more favorable course of CVHC.

Besides, the occurrence frequency of the wild type genotype of CYP2C9 (C/C) polymorphism in the population was studied, it made 75.3%. When comparing these data with literature data, there is a slight difference in the frequency of occurrence of genotypes (14). Thus, the frequency of occurrence of the "wild" genotype of CYP2C9 polymorphism in Turkey was 61.7% (6), in Malaysia - 94.0% (7), in Korea - 88.0% (8). A certain difference in the data obtained in the research and the literature data are apparently explained by the population characteristics of the studied groups.

The homozygous genotype for the mutant allele (T/T) was not determined either in the control or in the main group of patients with CVHC, which may indicate its low population incidence. All cases of mutant allele identification were represented by a heterozygous genotype (C/T). Moreover, in patients with chronic hepatitis C, the frequency of heterozygous genotype occurrence was 26.2%, which was comparable with the value of this indicator in the group of control patients (24.7%) with an unreliable difference in the result ($\chi 2 = 0.05$; $P = 0.82$; OR = 1.08; 95% CI0.5565-2.1). A similar control value of the heterozygous genotype frequency indicator was also noted in the subgroup of patients with cirrhosis (24.3%; $\chi 2 = 0.002$; $P = 0.97$; OR = 0.98; 95% CI 0.3966-2.423). At the same time, an increase in the frequency of occurrence of the C / T genotype was noted in the subgroup of patients with high active, and the lowest frequency indicator was observed in the second group of patients at the peak of the activity of the pathological process (16.2%; $\chi 2 = 1.06$; $P = 0.30$; OR = 0.59; 95% CI0.2151-1.62). Moreover, the difference in the incidence rate of the heterozygous allele between the second and other subgroups of patients was not significant.

The increased frequency of the C/T genotype in the group of patients with high active CVHC indicates that it may be associated with an inactive course of the disease. This is also confirmed by the tendency to a decrease in the frequency of the heterozygous genotype in the subgroup of patients with highly active chronic hepatitis C. However, the unreliability of the difference in the indicators does not allow us to unequivocally state the protective role of the C/T genotype with respect to the activation of the inflammatory process in CVHC and the connection of this genotype with a more favorable course of CVHC.

The increase in the frequency of the C/T genotype and simultaneously, the complete absence of homozygous genotype for the mutant T / T allele can be associated both with the selective advantage of the heterozygous 430C> T polymorphism of the CYP2C9 gene and the possibility of elimination of the mutant homozygous genotype.

A study of the frequency of polymorphism CYP2C9*3 gene 1075A> C alleles occurrence showed that in the population control group and in the patient subgroups, the wild-type allele was predominant. Moreover, the indicators were close in value (p> 0.05). Thus, in the control group, the frequency of occurrence of the “wild” allele of the studied polymorphism was 93.8%, in patients CVHC - 90.2%, in patients with high active CVHC - 86.4%, in patients with highly active chronic hepatitis C - 94.6%, in patients with cirrhosis of the liver - 89.2%.

A comparative analysis showed that in the main group of patients with chronic hepatitis C, the mutant C allele was more common (9.8%) - compared with the population control group (6.2%), but the difference was not significant. When analyzing the indicator of the frequency of the mutant allele in accordance with the stage of the disease and the activity of the pathological process, a certain range of values was noted. The highest frequency of the “C” allele among all the studied subgroups was detected in patients with high active chronic hepatitis C (HCV) (13.6%). The value of this indicator in the group of patients with cirrhosis was lower than in the first group (10.8%; $\chi 2 = 1.55$; P = 0.21; OR = 1.84; 95% CI0.70-4.88) , and the minimum frequency of the mutant allele was recorded in the group of patients with actively occurring CVHC. The increased frequency of the “C” allele of the 1075A> C polymorphism of the CYP2C9 gene in the group of patients with high active CVHB indicates its possible association with the inactive course of the disease. This assumption is also confirmed by the reduced frequency of the mutant allele in the subgroup of patients with highly active CVHC, which may indicate the protective role of this allele in relation to the activation of the inflammatory process in CVHC. Thus, there is a clear connection between the occurrence of the mutant allele of 1075A> C polymorphism of the CYP2C9 gene with a more favorable course of CVHC.

The frequency of occurrence of the wild type genotype of CYP2C9 polymorphism (A/A) in the studied population was 87.7%. When comparing these data with literature data in other populations, there is a slight difference in the frequency of occurrence of genotypes (9). It should be noted that in the study of the frequency of the 1075A> C polymorphism genotypes of the CYP2C9 gene in the present research, as in the case of the 430C> T polymorphism of the CYP2C9 gene, no homozygous genotype for the mutant allele (C/C) was determined in any of the groups, which most likely indicates its low population occurrence. All cases of detection of a mutant allele were represented by a heterozygous genotype (A/C). Moreover, in patients with CHVC, the frequency of occurrence of the heterozygous genotype was 19.6%, which was 1.6 times more than in the control

with an unreliable difference in the indicator (12.3%; $\chi 2 = 1.77$; P = 0.18; OR = 1.73; 95% CI 0.7667-3.92).

A certain range of values was noted when analyzing the frequency of the heterozygous genotype in accordance with the stage of the disease and the activity of the pathological process. The highest frequency of the A/C genotype among all the studied subgroups was identified in patients with high active CVHC (27.3%), and the minimum value of this indicator was noted in patients with an active pathological process - the frequency of the heterozygous genotype in these groups was 2.5 times. The frequency of the A/C genotype in patients with cirrhosis was 21.6% and did not have a significant difference with the other studied subgroups. The increased frequency of the A/C genotype in the group of patients with high active chronic hepatitis C is evidence of its possible association with the inactive course of the disease, which is also confirmed by the tendency to decrease the frequency of the heterozygous genotype in the subgroup of patients with highly active chronic hepatitis C. However, the unreliability of the difference in the indicators does not allow to unequivocally state the protective role of the C / T genotype with respect to the activation of the inflammatory process in chronic hepatitis C and the connection of this genotype with a more favorable course of CVHC.

The results showed the relationship between the mutant “C” allele and the heterozygous genotype containing this allele with more favorable course of CVHC, cannot be compared with literature data, since studies that have investigated the relationship of CYP2C9*3 polymorphism with the pathogenesis and progression of CVHC and the severity of liver lesion are not carried out.

Deviations from the equilibrium state of the 430C> T polymorphism of the CYP2C9 gene were also determined by the relative deviation of the expected heterozygosity from the observed or by the heterozygous deficiency index (index D). At the same time, a positive value of the index D means a deficiency of heterozygotes, a negative value means their excess.

Researching of the expected heterozygosity of the 430C> T polymorphism of the CYP2C9*2 gene in the studied groups of relatively healthy individuals and patients with CVHC showed that the h_{exp} value in the main group of patients was close to the control value (0.227 and 0.217; p> 0.05). Significant differences in the indicator were revealed between the subgroups of patients with CVHC. So, the highest level of expected heterozygosity was observed in patients with high active CVHC (0.316), while in patients with an active process the value of this indicator was 2.1 times lower (0.149; p> 0.05). In the group of patients with cirrhosis, the h_{exp} value was also lower than in the first group of patients, with an unreliable

difference in the indicator (0.214; p> 0.05). The results of the research suggest that a high level of theoretically expected heterozygosity is evidence of genetic diversity in a subgroup of patients with high active chronic hepatitis C. Moreover, in the group of patients with a highly active pathological process, genetic diversity is most limited - in comparison with other studied subgroups, which is characterized by a low rate of expected heterozygosity. However, the absence of significant intergroup differences in h_{exp} values may indicate that the development of chronic hepatitis C and the nature of disease course are not related to the level of heterozygosity.

Table 1

Difference between expected and observed frequency of heterozygosity polymorphism 430C>T of the CYP2C9*2 gene

Gene / polymorphism	Groups	Observed heterozygosity	Expected heterozygosity	D*	Reliability
Polymorphism 430C>T CYP2C9 gene	Core group	0,262	0,227	0,154	χ^2=2,42, p=0,12
	1-subgroup	0,394	0,316	0,247	χ^2=1,99, p=0,16
	2-subgroup	0,162	0,149	0,087	χ^2=0,29, p=0,59
	3-subgroup	0,243	0,214	0,135	χ^2=0,71, p=0,4
	Control group	0,247	0,217	0,138	χ^2=1,61, p=0,21

Observed heterozygosity of the 430C> T polymorphism of the CYP2C9 gene can judge the measure of genetic variation in the population. An analysis of the observed heterozygosity revealed that in the group of population control its value exceeded the expected heterozygosity (hobs = 0.247, h_{exp} = 0.217; p> 0.05). A small difference between the observed and expected heterozygosity was found in the main group of patients (hobs = 0.227, h_{exp} = 0.262; p> 0.05). Among the subgroups of patients, the highest value of hobs was noted in the group of patients with high active CVHC (0.394), and the lowest - in patients with highly active CVHC (0.162). The indicator of hobs in patients with cirrhosis of the liver had an intermediate value relative to the indicators of the first and second subgroups (0.243) and was close to the value of the control indicator (p> 0.05). This result may indicate that the sample of patients with a high proceeding process is

characterized by greater genetic variability, while the sample of patients with actively occurring chronic hepatitis C is less polymorphic.

Analysis of the expected heterozygosity showed that the h_{exp} value in the main group of patients was 1.5 times higher than the control value (0.177 and 0.116; p> 0.05). Some differences in the indicator were also revealed between the subgroups of patients with CVHC. So, the highest level of expected heterozygosity was observed in patients with high active CVHC (0.235), while in patients with an active process the value of this indicator was 2.3 times lower (0.102; p> 0.05). In the group of patients with cirrhosis, the h_{exp} value was also lower than in the first group of patients, with an unreliable difference in the indicator (0.193; p> 0.05). The results of the research indicate that a high level of theoretically expected heterozygosity is an indicator of genetic diversity in the subgroup of patients with high active chronic hepatitis C. Moreover, in the group of patients with a highly active pathological process, genetic diversity is most limited in comparison with other studied subgroups, as evidenced by the lowest value of expected heterozygosity. However, the absence of significant intergroup differences in h_{exp} values may indicate that the development of CVHC and the nature of the disease course are not related to the level of heterozygosity.

Conclusions. Thus, according to the research data, a relationship was established between the mutant allele of CYP2C9 gene polymorphism with a highly active CVHC. This assumption is proved by the reduced frequency of the mutant allele in the subgroup of patients with highly active CVHC. A high level of theoretically expected heterozygosity is the indicator of genetic diversity in the subgroup of patients with high active CVHC. The results obtained indicate that this marker can only conditionally be considered as a “protective” factor in relation to the progression of the disease and requires further study in a larger group of patients.

The obtained results showed the existence of differences in the frequency of occurrence of allelic variants of the studied polymorphisms in patients with favorable and unfavorable course of CHCV and various degrees of activity in the liver of pathological process caused by infection of CVHC. This fact proves that the disorders in regulation of the studied metabolism cytokines and enzymes of biotransformation production is a factor influencing the pathogenesis of CHCV.

References

1. Islambekova Z.A. Reducing the prevalence of viral hepatitis B and C in Uzbekistan - a component for the prevention of hepatocellular carcinoma // Oncology. - 2009. - T. 11, No. 2. - P. 154-157 (in Russian).
2. Perz J., Armstrong G., Farrington L. et al. The contribution of hepatitis B and hepatitis C viral infections to cirrhosis and primary liver cancer worldwide. // J. Hepatol., 2006.-№45:- P.529-538.
3. Bosch F.X.,Ribes J., Cleries R. et al. Epidemiology of hepatocellular carcinoma// Clin Liver Dis.–2005. – Vol. 9, №2. – P. 191-211.
4. Shepard C.W., Finelli L., Alter M.J. Global epidemiology of hepatitis C virus infection.// Lancet Infect Dis .-2005.-№5.-P.558-567.
5. Pilotto A., Seripa D., Franceschi M. et al. Genetic susceptibility to nonsteroidal anti-inflammatory drug-related gastroduodenal bleeding: role of cytochrome P450 2C9 polymorphisms.// Gastroenterology.-2007.- №133.-P.465-471.
6. Aynacioglu A.S., Brockmoller J., Bauer S., et al. Frequency of cytochrome P450 CYP2C9 variants in a Turkish population and functional relevance for phenytoin // Br. J. Clin. Pharmacol. –1999. –Vol. 483. –P.409-415.
7. Seng K.C., Gin G.G., Sangkar J.V., Phipps M.E. Frequency of Cytochrome P450 2C9 (CYP2C9) Alleles in Three Ethnic Groups in Malaysia // Asia Pacific J. of Mol. Biol. and Biotech. –2003. –Vol 1. –P.83-91.
8. Bae J.-W., Kim H.-K., Kim J.-H. et al. Allele and genotype frequencies of CYP2C9 in a Korean population // Br. J. Clin. Pharmacol. –2005. –Vol. 60. –P.418–422.
9. Sychev D.A., Stasyak E.V., Ignatiev I.V. et al. Clinical pharmacogenetics of the isoenzyme of cytochrome P-450 2C9 // Klin.pharmacol. and ter. - 2005.-No. 4.- P.60–63 (in russian).
10. Blackard J. N., Shire N.J. et al. Acute hepatitis C virus infection:a chronic problem//Hepatology.- 2008.– Vol. 47. – P. 321-331
11. Albeldawi et al. Hepatitis C virus: prevention, screening, and interpretation of assays. ClevelClin// J. Med.– 2010. – Vol. 77. – P. 16–26.
12. Gochee P.A., Jonsson J.R., Clouston A.D., et al. Steatosis in chronic hepatitis C: association with increased messenger RNA expression of collagen I, tumor necrosis factor-alpha and cytochrome P450 2E1 // J Gastroenterol Hepatol. –2003, Apr. –18(4). –P.386-392.
13. Frye R.F.,Zgheib N.K.,Matzke G.R., et al. Liver disease selectively modulates cytochrome P450--mediated metabolism // Clin Pharmacol Ther.–2006 Sep.–Vol.80, №3. –P.235-245.

14. Carlquist J., Horne B., Muhlestein J. at al. Genotypes of the cytochrome p450 isoform, CYP2C9, and the vitamin K epoxide reductase complex subunit 1 conjointly determine stable warfarin dose: a prospective study // Journal of Thrombosis and Thrombolysis. – 2006. – Vol. 22. –P.191-197.